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BIOTA OF FRESHWATER ECOSYSTEMS. IDENTIFICATION MANUAL NO. 10. GENERAL OF FRESHWATER NEMATODES (NEMATODA) OF EASTERN NORTH AMERICA

V. R. Ferris, et al

Purdue University

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Biota of Freshwater Ecosystems

Identification Manual No. 10

GENERA OF FRESHWATER NEMATODES (NEMATODA) OF EASTERN NORTH AMERICA

by

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for the

ENVIRONMENTAL PROTECTION AGENCY

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FOREWORD

"Genera of Freshwater Nematodes (Nematoda) of Eastern North America" is the tenth of a series of identification manuals for selected taxa of invertebrates occurring in freshwater systems. These documents, prepared by the Oceanography and Limnology Program, Smithsonian Institution for the Environmental Protection Agency, will contribute toward improving the quality of the data upon which environmental decisions are based.

Additional manuals will include but not necessarily be limited to, freshwater representatives of the following groups: branchiuran crustaceans (*Argulus*), amphipod crustaceans (Gammaridae), isopod crustaceans (Asellidae), decapod crustaceans (Astacidae), leeches (Hirudinea), polychaete worms (Polychaeta), freshwater planarians (Turbellaria), dryopoid beetles, and freshwater clams (Sphaeriacea).

ABSTRACT

An illustrated key to 56 genera of freshwater nematodes of eastern North America is given. Notes are included on the significance of nematodes in freshwater ecosystems, collecting and isolating nematodes, slide preparations and counting, and identification and use of the key.

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SECTION I

INTRODUCTION

Fifty-six genera of freshwater nematodes are included in this key. It is reasonable to assume that knowledge about such a large group of animals would be important and useful to the understanding of freshwater ecosystems. Nevertheless, nematodes have been overlooked or avoided by most aquatic biologists, probably because they are small and somewhat difficult to handle. They are frequently very numerous, although their total biomass may be relatively low because of their small size. However, their role in aquatic ecosystems may be much greater than their biomass would indicate since they have a very high metabolic rate.

Nematodes are found in soil and marine habitats as well as all freshwater habitats. Some nematodes are capable of living in both soil and freshwater whereas others are found only in freshwater or only in soil. The orders Tylenchida and Dorylaimida are primarily found in soil and have only a few freshwater representatives. Other orders of nematodes are better represented in freshwater than in soil, and are even more richly represented in marine habitats. Parasitic nematodes occur on or in many aquatic animals, but are not included in this key, as they are usually not collected in the same manner as free-living nematodes or nematodes parasitic on plants.

The only major systematic publications on North American aquatic nematodes are those of N.A. and M.V. Cobb (1913, 1914, 1915). However, samples of aquatic nematodes seldom yield species which cannot be placed in a known genus, although undescribed species may be very common. Many of the genera of aquatic nematodes are very widely distributed, and at least some may be cosmopolitan in distribution.

Nematodes feed on a wide variety of organisms, but apparently do not feed on dead organic matter. Stylet-bearing nematodes feed on many types of higher and lower plants and on small animals by puncturing them and drawing out the liquid contents. Nematodes with simple, unarmed stomata probably feed mainly on small unicellular organisms such as bacteria. Some nematodes have large teeth which they use to attack other small animals including other nematodes.

A recent study in our laboratory (Ferris et al., 1972) has indicated that an increased understanding of disturbances to aquatic habitats can be obtained by a study of nematode communities of the habitats. In our study of small freshwater streams, nematode species of the genera *Monhystera*, *Mesodorylaimus* and *Tylenchus* proved to be especially numerous in certain of the stream sites, with species of the genera *Acrobeloides*, *Tobrilis*, *Mononchoides* and *Goffartia* also numerous at some sites.

It is hoped that this key will make possible more extensive use of data on nematode community structure by persons concerned with evaluating water resource environments. This kind of analysis can be a useful and practical tool, particularly when used in combination with other available techniques, for interpreting ecological conditions and providing indices of change.

COLLECTING AND ISOLATING AQUATIC NEMATODES

Nematodes can be found in or on all kinds of benthic substrates. Since they are too small to be collected individually in the field, samples of substrate are usually taken to the laboratory for processing. Nematodes may also be found in small numbers floating in water even though they are primarily associated with benthic material. Floating nematodes have been collected by passing water through fine screens (U.S. Standard Series #400 sieve), the nematodes and associated debris accumulating on the screen (Faulkner and Bolander, 1966).

Nematodes must be separated from all particulate matter from their habitat before they can be examined. Even tiny particles of soil or debris will obscure the morphologic details of these microscopic organisms. They may be isolated by picking them out of small samples of substrate using a finely pointed bamboo or nylon needle ("pick") while observing the manipulations under a stereoscopic microscope. However, hand sorting is used when only a few nematodes are needed since it is very time consuming.

During the processing of large samples (500-1000 cc) of particulate matter consisting of silt, clay, sand and organic matter, nematodes may be partially separated from this debris by a combination of decanting and sieving. To process a sample in this manner, soil or sediment is mixed with water, allowed to stand for 30 seconds to allow heavier particles to settle, and the supernatant (containing the nematodes) is poured through a sieve. Various sizes of sieves are used to remove nematodes from the water. All aquatic nematodes will pass through a U.S. Standard Series #10 sieve, so this size is used to remove floating organic matter. Very large nematodes (2-4 mm in length) will collect on a #25 sieve. A #325 sieve will catch the smallest (0.5-0.1 mm) nematodes unless they pass through head or tail first. By passing water containing suspended nematodes through a #325 sieve several times, almost all the nematodes will be caught. Processing soil in this manner will not separate the nematodes from all particles, but it does concentrate them so that they can be more effectively isolated with a Baermann funnel. A Baermann funnel is made by slipping a short piece of rubber tubing on the stem of a 100-150 mm funnel. The tubing is closed with a Day-type pinch clamp so that the funnel will hold water. Nematodes and substrate are placed in the water on top of a paper handkerchief (any commercial brand paper handkerchief or tissue which possesses sufficient wet-strength not to disintegrate may be used) or muslin square supported by a wire basket in a funnel filled with water to a level that just covers the sample. Twenty-four hours later the nematodes which have fallen into the stem of the funnel after migrating through the tissue or muslin can be removed by opening the pinch clamp and collecting 5-10 ml of water. Very small samples of soil or samples of substrate such as twigs or small stones with nematodes on their surfaces can be placed directly in Baermann funnels to isolate the nematodes.

Steps used in isolating nematodes by decanting, sieving and the Baermann funnel may vary, but the following procedure, used in our laboratory, works well for routine mass collections:

Materials:

2 ten quart buckets labelled "A" and "B"
2 flat pans 18-25 cm diam. X 6-8 cm high
3 250 ml beakers
2 Baermann funnels for each sample processed
1 series of U.S. Standard Series sieves: #10, #25, #100, and #270

Steps:

1. Place 500-1000 cc of sediment or soil in bucket A. Fill bucket one-fourth or less with water and break up all lumps. Allow material to stand 30 seconds and then decant supernatant through the #10 sieve into bucket B. Repeat this operation, washing all fine particles from the coarse material in bucket A, until bucket B is filled to within 5-10 cm of the top. Discard material remaining in the bottom of bucket A and on the #10 sieve. Rinse bucket A to clean.

2. Pour the contents of bucket B through a #25 sieve, catching the water passing through the sieve in bucket A. Decanting must be stopped before large soil particles collect on the sieve. Invert the sieve and flush material caught on the sieve into one of the pans. After a short settling period, pass the water in the pan through the sieve again and catch this water in bucket A. Rinse the residue on the #25 sieve into a clean pan using 250 ml of water or less. Then pour the contents of the pan into a 250 ml beaker and set it aside to permit nematodes to settle to the bottom. Rinse bucket B to clean.

3. Repeat step 2 using a #100 sieve, pouring the contents of bucket A through the sieve and catching the water again in bucket B. Wash the residue on the #100 sieve into a pan, then pass the contents of the pan through the #100 sieve again (catching this water in bucket B). Re-suspend material caught on this sieve in 250 ml of water or less and place in a second beaker, to allow all nematodes to settle to the bottom.

4. To obtain the remainder of the nematodes, pour the contents of bucket B through the #270 sieve, catching the water in bucket A. After washing the material caught on the #270 sieve into a pan, pass the water in bucket A through the #270 sieve again (this water may now be discarded). Wash the material on the sieve into the same pan used for the first material caught on the #270. In

all instances, pouring from a bucket through a sieve should be stopped before the silty material in the bottom of the bucket is decanted onto the sieve. This remaining silt is discarded when the bucket is rinsed.

5. The nematodes, now in the water in the pan, are further concentrated by pouring the water through the #270 sieve, catching it in the second pan. Repeat this procedure two more times, each time pouring the water through a different area of the same #270 sieve. The residue now caught on the sieve is washed off the sieve and back into the pan using 250 ml or less of water. Allow this to settle 30 seconds and decant into a third 250 ml beaker where nematodes will settle to the bottom.

6. After the contents of the three 250 ml beakers have settled (for about an hr.) carefully decant the supernatant.

7. Pour some of the residue from the beaker in which material from the #25 sieve was saved into a Syracuse watch glass or other flat-bottomed dish and examine with a stereoscopic microscope at 30-60 X magnification. Large nematodes may be picked out of the dish with a finely pointed pick and transferred to a vial of water. Examine all the material from this beaker, picking out all the large nematodes observed.

8. To complete the nematode separation procedures, the residues from the #100 and #270 sieves (now in beakers) are each placed separately on tissue or muslin in Baermann funnels, in the manner described previously. Nematodes are removed from the funnels at 24 and 48 hours by opening the pinch clamp and drawing off 5-10 ml of fluid into a vial. It may be necessary to add additional water to the funnel to keep the material submerged. The nematodes should now be free of silt and organic matter, ready for preservation and observation.

Other methods for extraction of nematodes are available, and are described in various nematology texts (Thorne, 1961; Southey, 1970).

TECHNIQUES FOR PREPARING NEMATODE SLIDES AND FOR COUNTING

Identification of nematodes usually requires they be mounted on slides. They may be mounted temporarily in water or fixative, or permanently in glycerin. Permanent mounts should be used when extensive study is contemplated. It is not necessary to stain nematodes for identification procedures.

In order to preserve nematodes, they must first be killed by gentle heat. Such a killing procedure is necessary because live nematodes placed directly in fixative become distorted. Nematodes may be killed by placing the vial containing them in a water bath at 57°C for 10 minutes, or in an oven at 52°C for 15 minutes. If an oven or water bath is not available, nematodes may be heat relaxed by adding a quantity of boiling water to an equal quantity of water at room temperature in a beaker containing the nematodes to be killed. After heat relaxing, the nematodes should immediately be placed in fixative. The fixative may be warmed so that it is about the same temperature as the water containing the killed nematodes. Many different fixatives are available, but a commonly used one is 5% formalin. For fixing, and also for storing mass collections, a 10% solution of formalin is added to an equal quantity of water containing killed nematodes, so that the final concentration of formalin is 5%. Nematodes for permanent mounts should be fixed in F.A.A. (8 ml commercial 37% formaldehyde solution, 1 ml glacial acetic acid, 20 ml 95% ethanol, and 50 ml H_2O) for at least two weeks. This period of time insures proper fixation of fine, definitive morphological details.

Temporary mounts can be made by transferring freshly killed nematodes to a drop of 5% formalin on a slide and placing a cover glass on the drop. The drop of formalin should not be so large that excessive amounts of fluid run out from under the cover glass, or so small that air pockets form under the cover glass. The nematodes should be at the bottom and middle of the drop on the slide, and three glass rods, each approximately 1 mm long, should be arranged around them before the cover glass is lowered. The glass rods, about the same diameter as the nematodes to be mounted, prevent the cover glass from flattening the nematodes. A selection of various sized fine glass rods can be obtained by heating rods of soft lead glass over an alcohol burner and pulling them apart. To produce very fine rods, hot glass must be drawn out quickly; slower pulling produces rods of greater diameter. Glass wool or angel hair may contain the right diameter fibers for some nematodes.

When making temporary mounts, the cover glass should be lowered slowly to prevent the nematodes from moving to the edge. Next the cover glass should be tacked down with small drops of ringing compound at several points around the edge. After these tacks have dried, the remainder of the cover glass is sealed to the slide with more ringing compound. Temporary slides can be sealed with wax by lighting a small candle,

putting it out, and applying the hot wax using the wick of the candle as a brush. Clear finger nail polish may be used as a ringing material. A special ringing compound, called Zut (available from Bennett Paint Products, Salt Lake City, Utah), is commonly used by nematologists for ringing temporary as well as permanent slides. Zut is thinned with butyl acetate to a consistency which is easy to apply with a #3 or finer camel's-hair brush. However, it should be thick enough to give a good seal. Brushes may be cleaned with butyl acetate.

For permanent mounts, nematodes previously fixed in F.A.A. are infiltrated with glycerin. To do this, place the nematodes in an alcohol-glycerin mixture which contains 1 1/2% glycerin (3 ml glycerin, plus 50 ml ethanol, plus 147 ml H_2O). Allow the water and alcohol to evaporate off slowly over a period of about four weeks. If the desiccation process takes place too rapidly, the nematodes will collapse. A convenient way to control the dehydration is to place the 1 1/2% glycerin solution containing the nematodes in a small watch glass which holds about 2 ml of fluid. At the start of the dehydration-infiltration, the watch glass should be filled to the top with the dilute glycerin mixture. The watch glass can be placed in a container such as a preparation dish. A desiccant such as calcium chloride in a small screw-cap vial with a small hole drilled in it is placed in the preparation dish with the watch glass. Petroleum jelly should be applied between the rim and the top of the dish to seal it. We have obtained good results by placing about a dozen of the small watch glasses in a square plastic refrigerator-storage container (sandwich-size) with a very tight lid. One vial of desiccant prepared as described above is placed in the container with the watch glasses. At the end of the dehydration period, only a thick film of glycerin with nematodes remains in the bottom of the watch glass. Infiltrated nematodes should be stored in a desiccator since glycerin readily absorbs moisture from the atmosphere.

Permanent slide mounts are made with glycerin-infiltrated nematodes in a fashion similar to that described above for temporary mounts. From 1-6 nematodes of like diameter are selected for mounting on a single slide. Special care should be taken in selecting the glass rods for supporting the cover glass and in using the correct amount of glycerin. If the rods are larger in diameter than the nematodes, or if too much glycerin is used, the nematodes will float under the cover glass, making observations with the oil immersion objective of a compound microscope extremely difficult. On the other hand, if the diameter of the rods is too small, the nematodes will be flattened and distorted.

Arrange the nematodes carefully in the center and bottom of a drop of glycerin with their heads all pointed in the same direction. Use glycerin which has been stored in a desiccator. Arrange three glass rods around the nematodes. Warm a cleaned cover glass over an alcohol lamp and lower it slowly onto the glycerin. Tack the cover down with Zut. After the tacks have dried, ring the cover glass with more Zut. Nematode slides should not be stored resting on their edges.

Cobb metal slide mounts are often used by nematologists for mounting nematodes. These mounts do not break as easily as glass slides and have the added advantage that they can be stacked and the ringing material of one slide does not touch the one next to it. In a Cobb mount, the nematodes are mounted between two cover glasses, and thus can be examined with an oil immersion objective from either the top or bottom of the slide. A Cobb slide consists of a 25 mm square #1 cover glass held in place over a round hole (18 mm diameter) in the center of an aluminum 75 X 25 mm slide (with rolled edges) by two pieces of cardboard (Mason and Bosher, 1963). An 18 mm circular cover glass is placed on top of the square glass over the hole in the metal slide. After the Zut has dried, the edges of the Cobb mount are crimped over the cardboard to hold the square cover glass firmly in place. (See Thorne (1961) for more details regarding techniques for making slides).

For identification of nematode species in an area under study, slide mounts should be prepared by one of the methods described above. After initial identification, however, most species can be recognized subsequently at magnifications available on low-power stereoscopic microscopes (e.g. 30 X and 60 X). This makes possible the counting of individuals of dominant species (obtained from substrate samples of standardized sizes) in a petri dish marked off in squares or lines. If the sample contains too many individuals for accurate counting, a measured portion or aliquot may be drawn off and additional water or fixative added before counting the nematodes in the aliquot. The number of individuals in the entire sample is calculated based on the size of the aliquot and the numbers of individuals actually counted. Most nematologists prefer to count more than one aliquot to increase the accuracy of the population estimate.

IDENTIFICATION AND USE OF THE KEY

The basic body shape of nematodes is an elongate cylinder with the oral opening at the anterior end. These organisms are internally nonsegmented, although thickenings of the cuticle may give the appearance of rings or body segmentation. Several of the sediment inhabiting nematode genera have readily observed amphids (Fig. 1 A-F), sensory organs located behind the lips, which are of importance in identification procedures. The stoma ("mouth") of a nematode, intimately in contact with its food source, shows a variety of forms and modifications which are used to diagnose genera. The esophagus is located in the area between the stoma and the intestine. Its form and shape (Fig. 2 A-L) is also of diagnostic importance. The anus, ventral and sub-terminal on nematodes, serves as a demarcation point for the region referred to as the "tail". Thus the tail is that portion of a nematode posterior to the anus. The tail shape (Fig. 3 A-G) is often used to separate nematode genera. Nematodes are biparental with the sexes differing primarily in their secondary sexual characters: one or two ovaries and a vulva in the female; one or two testes, one or two spicules, a bursa (not always present) and a cloaca in the male.

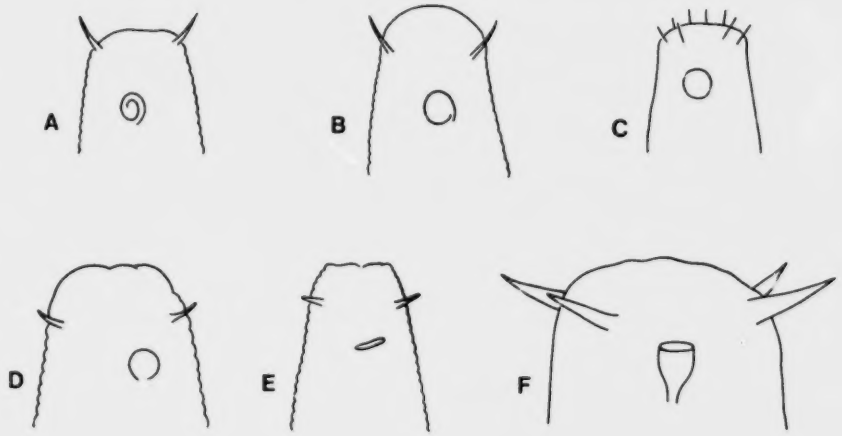

Fig. 1 - Amphid shapes: A, head, *Achromadora* sp. (multispiral); B, head *Prodesmodora* sp. (unispiral); C, head *Monhystera* sp. circular); D, head *Plectus* sp. (open circle); E, head *Anaplectus similis* (slit-like); F, head *Tobrilus* sp. (stirrup-shaped); (all X 1000).

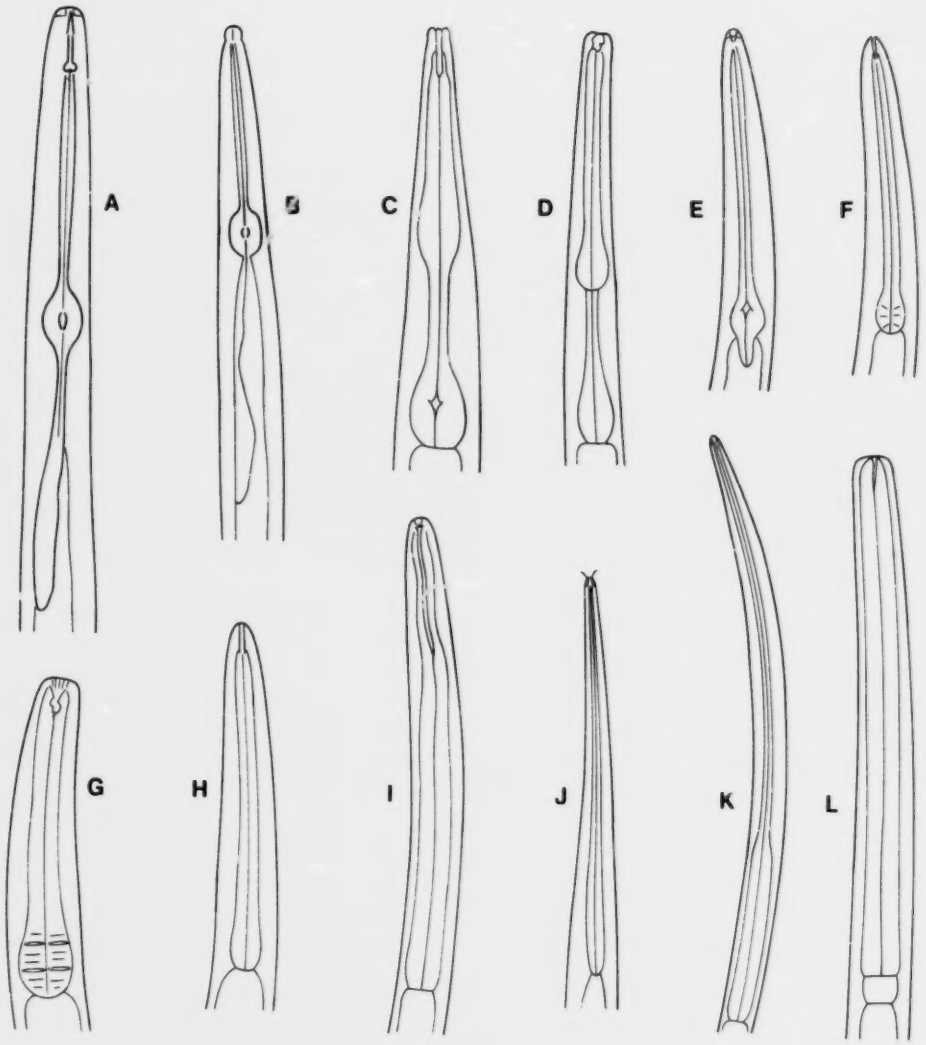

Fig. 2 - Esophagus shapes: A, *Hirschmanniella* sp. (X 500); B, *Aphelenchoides* sp. (X 500); C, *Rhabditis* sp. (X 500); D, *Butlerius* sp. (X 250); E, *Plectus* sp. (X 250); F, *Leptolaimus* sp. (X 500); G, *Achromadora* sp. (X 500); H, *Cylindrolaimus* sp. (X 500); I, *Ironus* sp. (X 250); J, *Aphanolaimus* sp. (X 250); K, *Alaimus* sp. (X 250); L, *Tripyla* sp. (X 250).

Whenever possible, difficult or ambiguous characters have been avoided in this key. In general, the diagnostic characters used in this key are illustrated with line drawings of representative species. Frequently two or three characters are used in a couplet, instead of a single character, to give more confidence in making identifications and also to provide more information about the traits of each genus. Definitions for terms which may not be familiar to the general biologist are given in a glossary (p. 35).

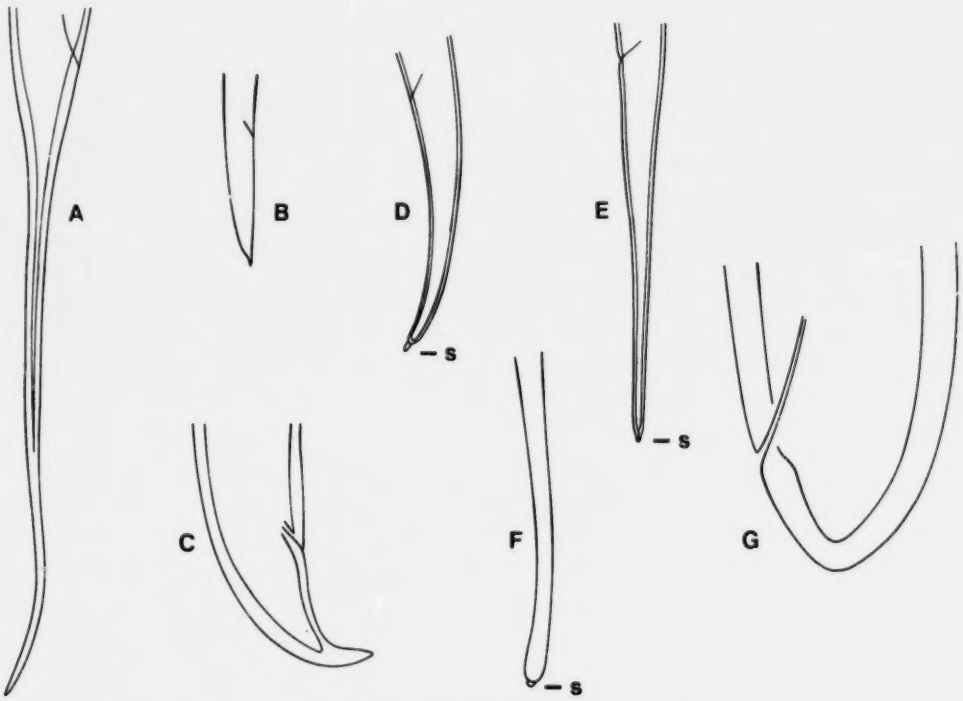

Fig. 3 - Tail shapes: A, *Mesodorylaimus* sp.; B, *Aphelenchoides clarus;* C, *Eudorylaimus meridionalis;* D, *Achromadora* sp.; E, *Tobrilus* sp.; F, *Monhystera* sp.; G, *Labronema thornei* (all X 500). *s*=spinneret.

Occasionally a specimen may be encountered which cannot be identified using this key because it is an unusual form of a known genus or it belongs to an undescribed genus. Such specimens will probably be rare since the nematode genera of northeastern United States are fairly well known. To be certain of identification, specimens should be compared with figures in the key, and if any doubt remains, they should be checked against complete descriptions, or they may be sent to an expert for verification. Many genera of terrestrial nematodes (which are often washed into aquatic habitats) are not treated in this key, so other references must be used if identification to genus of these specimens is desired. Two orders (Tylenchida and Dorylaimida) and two families (Rhabditidae and Diplogasteridae) which contain many of the genera of terrestrial nematodes are end points in the key. For further information on the Tylenchida and Dorylaimida, refer to Zuckerman, Mai and Rohde (1971).

The book "Soil and Freshwater Nematodes" (Goodey, 1963) may be used as a general reference since it contains descriptions of almost all genera of terrestrial and aquatic nematodes. However, there are two exceptions. *Laimydorus* Siddiqi, 1969, was described after publication of the Goodey book, and *Hirschmanniella* is listed as *Hirschmannia* in Goodey. This book has a fairly extensive list of references for soil and aquatic nematodes. The book "Principles of Nematology" by Thorne (1961) is an especially good reference for soil forms. For more extensive listings of taxonomic works, the check lists of Tarjan (1960, 1967) and Baker (1962) should be consulted. Helminthological Abstracts is a good source for references dealing with all phases of nematology.

Identification of aquatic nematodes to species is usually difficult even for experts, and is complicated by the occurrence of undescribed species. Since an extensive literature file is required for species identification, it is suggested that material be sent to a nematode taxonomist, if it is considered essential that a specific name be given to a specimen. Nematologists who have recently published descriptions of aquatic or free-living nematode species are good candidates for this service. Needless to say, as long as a species is recognizable, much valuable ecological information may be obtained from it, even though it is referred to by a code designation rather than a Latin name. In the interests of accurate science, preserved specimens should be retained of all species considered in any data used in publication.

A list is appended to the key which places each genus in an order and suborder of nematodes. The classification is based on that of de Coninck (1965) and differs somewhat from that of Goodey (1963). Families are not included because of the frequent changes now occurring in this taxon.

SECTION II

KEY TO GENERA OF NEMATODES OF EASTERN NORTH AMERICA

1 Stoma large, cup-shaped; width and depth of stoma at least one-half of lip region; stoma strongly cuticularized (Fig. 4 A, B, C: 5 D) 2
Stoma not both wide and cup-shaped and either weakly or strongly cuticularized 11

2(1) Cephalic setae present (Fig. 4 A, B) 3
Cephalic setae absent (setose papillae may be present) 4

3(2) Tail elongate-clavate with spinneret; 4 large cephalic setae present (Fig. 4 A): *Anonchus*
Tail filiform and lacking spinneret; 6 large and 4 short cephalic setae present (Fig. 4 B): *Prismatolaimus*

4(2) Esophagus with median and posterior bulbs (Fig. 2 D) ....Diplogasteridae..... 5
[Several genera in this family, in addition to those included in this key, may occasionally be found in aquatic habitats. See Goodey (1963) for names of genera and illustrations.]
Esophagus cylindrical; no median bulb 6

5(4) Stoma very broad and deep; anterior edge of stoma not marked and not bearing grooves or rib-like structure (Fig. 4 C): *Butlerius*
Stoma deep and moderately broad; anterior edge of stoma bearing rib-like structures; slender tubular section of stoma extending posterior to large tooth in stoma (Fig. 4 D): *Mononchoides*

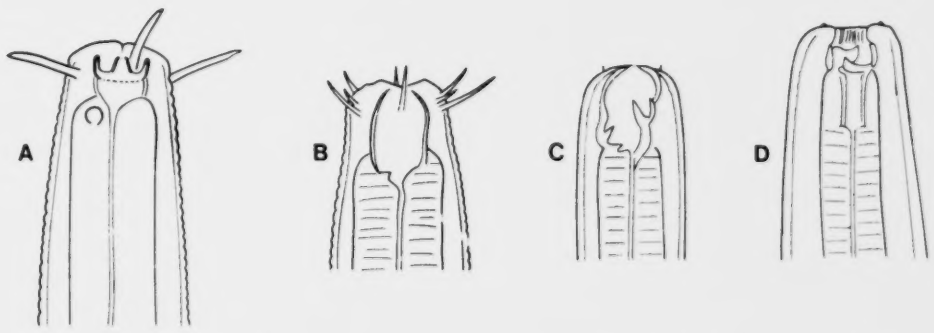

Fig. 4 - A, head *Anonchus* sp.; B, head *Prismatolaimus* sp.; C, head *Butlerius* sp.; D, head *Mononchoides* sp.; (all X 1000).

6(4) Stoma with large subventral tooth; dorsal tooth obscure (Fig. 5 B); spinneret on ventral portion of tail (Fig. 5 C): *Mononchulus*
Stoma with large dorsal tooth; size of subventral teeth variable; spinneret at terminus of tail 7

7(6) Dorsal tooth posteriorly directed in stoma (Fig. 5 A): *Anatonchus*
Dorsal tooth anteriorly directed in stoma 8

8(7) Large dorsal tooth posteriorly placed in stoma; two large subventral teeth opposite to dorsal tooth (Fig. 5 D): *Miconchus*
Dorsal tooth anteriorly placed in stoma; subventral teeth absent or not large 9

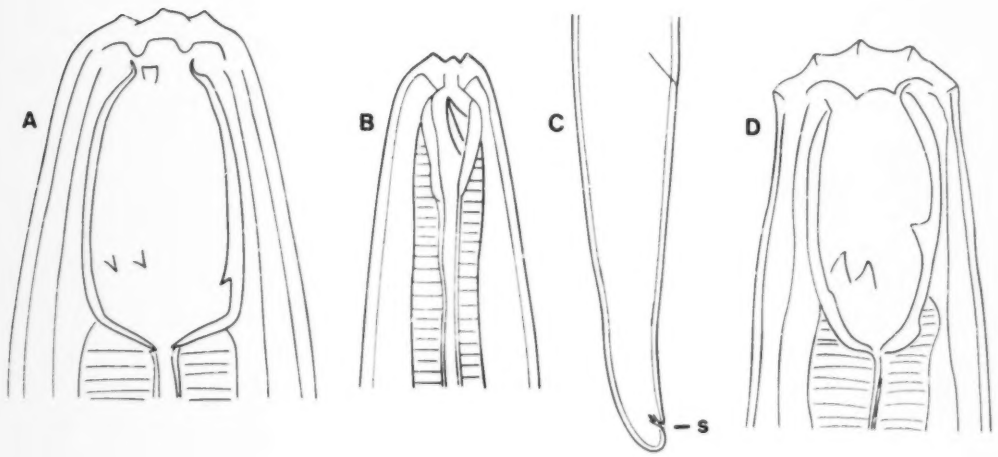

Fig. 5 - A, head *Anatonchus* sp. (X 500); B, head *Mononchulus* sp. (X 1000); C, female tail *Mononchulus* sp. (X 500), *s*=spinneret; D, head *Miconchus trionchus* (X 500).

9(8) Stoma with transverse row of denticles opposite to dorsal tooth (Fig. 6 I): *Mylonchulus*
No transverse row of denticles opposite dorsal tooth 10

10(9) Longitudinal ridge without denticles opposite dorsal tooth (Fig. 6 B): *Mononchus*
Longitudinal row of denticles opposite dorsal tooth (Fig. 6 A): *Prionchulus*

11(1) Stoma armed with spear (Fig. 6 H; 7 C, F; 8 A) or spear-like tooth (Fig. 6 C) 12
Stoma lacking spear or spear-like tooth 26

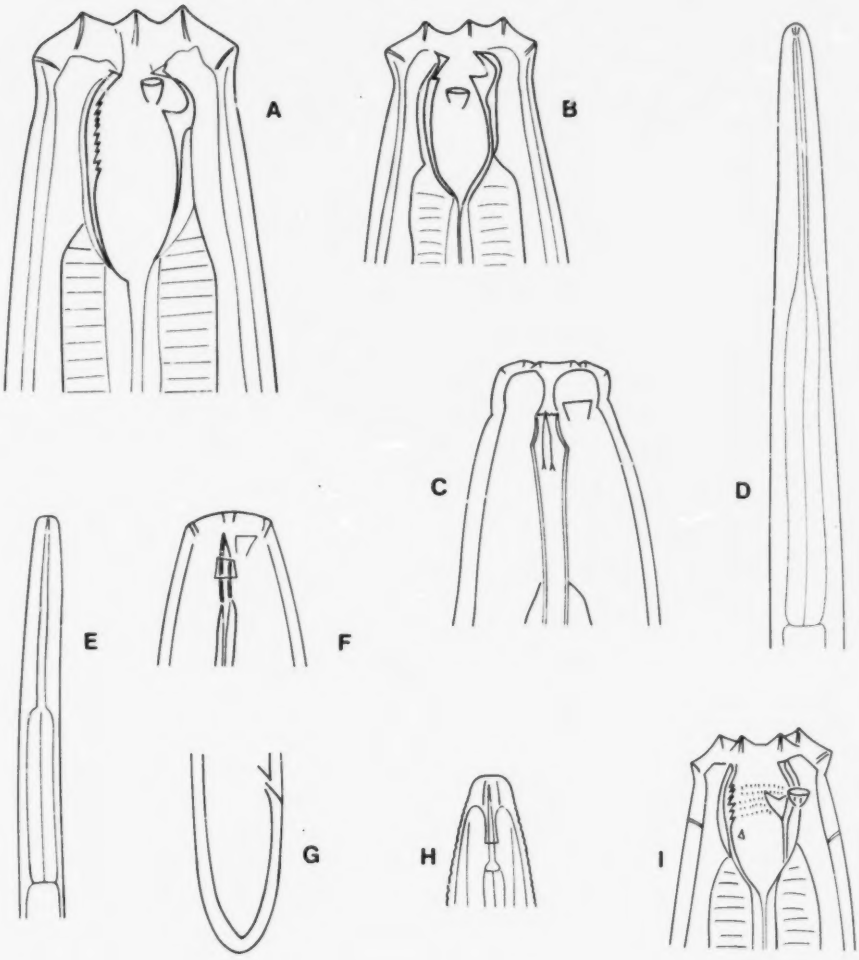

Fig. 6 - A, head *Prionchulus punctatus* (X 750); B, head *Mononchus papillatus* (X 750); C, head *Nygolaimus* sp. (X 1000); D, anterior part *Nygolaimus* sp. (X 250); E, anterior part *Thornia* sp. (X 250); F, head *Thornia* sp. (X 1000); G, female tail *Thornia* sp. (X 500); H, head *Tylenchus cylindricus* (X 1000); I, head *Mylonchulus brachyuris* (X 750).

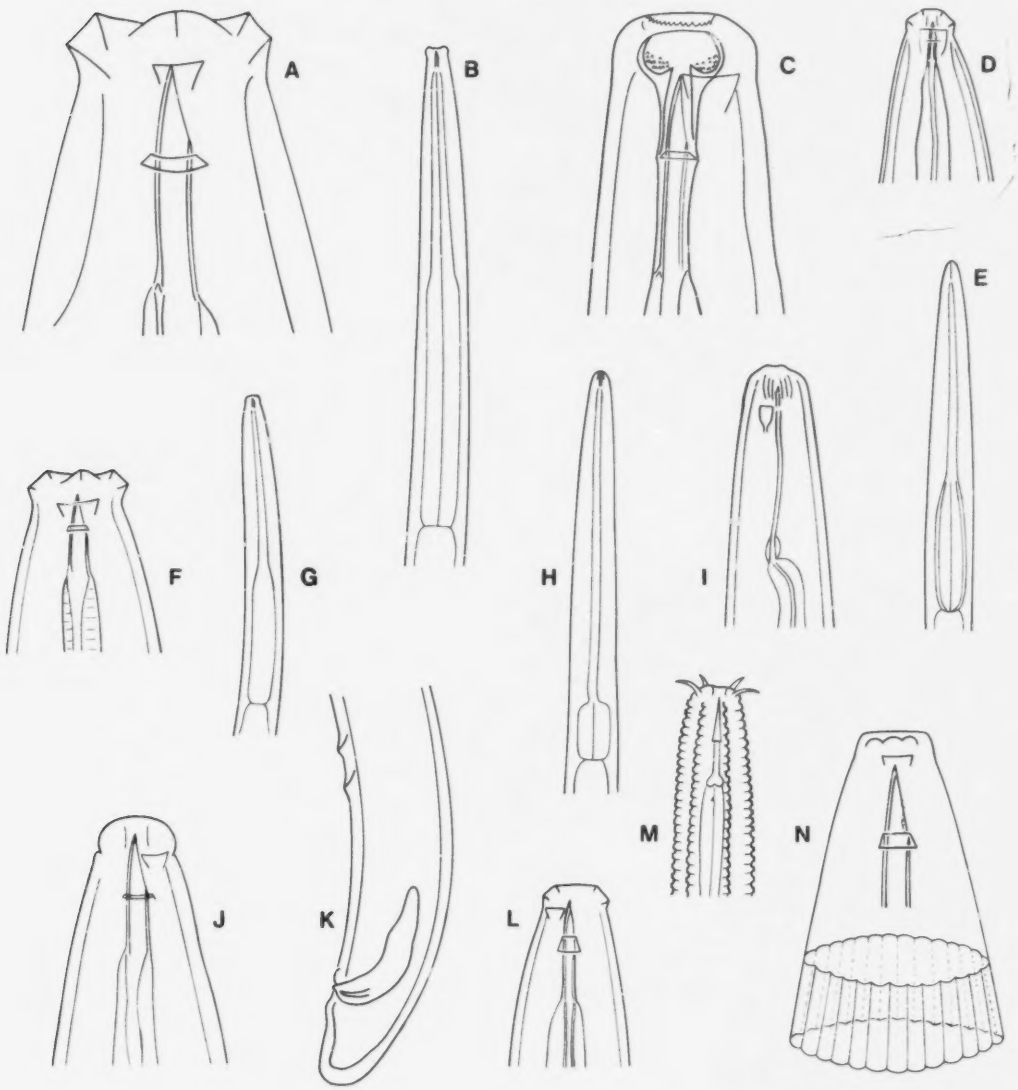

Fig. 7 - A, head *Labronema thornei* (X 1000); B, anterior part *Labronema thornei* (X 100); C, head *Paractinolaimus* sp. (X 1000); D, head *Oxydirus oxycephalus* (X 1000); E, anterior part *Oxydirus* sp. (X 250); F, head *Eudorylaimus meridionalis* (X 1000); G, anterior part *Eudorylaimus meridionalis* (X 100); H, anterior part *Aulolaimoides elegans* (X 250); I, head *Aulolaimoides elegans* (X 500); J, head *Mesodorylaimus* sp. (X 1000); K, male tail *Mesodorylaimus* sp. (X 500); L, head *Laimydorus* sp. (X 500); M, head *Atylenchus* sp. (X 1000); N, head *Dorylaimus* sp. (X 500).

12(11) Esophagus lacking median bulb; amphid stirrup-shaped and fairly distinct. Dorylaimida..... 13
Esophagus with median bulb; amphid obscure. Tylenchida.... 22
[Several genera in these orders which are not truly aquatic and are not included in this key, may be found in aquatic habitats and may be carried into aquatic situations for which they are not adapted. For further information on such genera and illustrations see Thorne (1961) and Goodey (1963).]

13(12) Tails of both sexes short and blunt or elongate, but not filiform (Fig. 3 C, G) 14
Female or both female and male tail filiform (Fig. 3 A) ... 17

14(13) Tail blunt and approximately twice as long as the anal body diameter (Fig. 6 G); anterior end of body nearly square (Fig. 6 F); esophagus expands very sharply at middle (Fig. 6 E): *Thornia*
Tail pointed or if blunt then distinctly shorter than twice anal body diameter; anterior end of body somewhat rounded; esophagus expands gradually 15

15(14) Tooth-like spear attached to stoma wall; stoma wall cuticularized and well separated from tooth (Fig. 6 C); esophagus often expanded anterior to middle (Fig. 6 D): *Nygolaimus*
Spear present, located centrally in stoma; walls of stoma weakly developed and barely visible; esophagus usually expanded at middle 16

16(15) Anterior portion of esophagus considerably narrower than posterior portion (Fig. 7 G); total length usually 2 mm or less (Fig. 7 F): *Eudorylaimus*
Anterior of esophagus nearly as wide as posterior portion (Fig. 7 B); total body length usually greater than 2 mm (Fig. 7 A): *Labronema*

17(13) Enlarged basal portion of esophagus short, barely longer than wide (Fig. 7 H); weakly developed spear surrounded by faint cuticularized ribs (Fig. 7 I): *Aulolaimoides*
Basal portion of esophagus considerably longer than wide; spear not surrounded by ribs 18

18(17) Four large teeth or thick cuticularized framework surrounding spear; denticles present in cavity around spear (Fig. 7 C): *Paractinolaimus*
No large teeth or thick cuticularized framework surrounding spear; no denticles in cavity around spear 19

19(18) Esophagus surrounded by muscular sheath (Fig. 7 E); spear length approximately equal to lip width (Fig. 7 D); both male and female tail filiform: *Oxydirus*
Esophagus not surrounded by muscular sheath; spear longer than lip width; male tail short and blunt (Fig. 7 K) 20

20(19) Cuticle with longitudinal ridges (Fig. 7 N); total body length greater than 2 mm: *Dorylaimus*
Cuticle without longitudinal ridges; total body length greater or less than 2 mm 21

21(20) Total length usually less than 2 mm; spear guiding ring usually single (Fig. 7 J): *Mesodorylaimus*
Total length usually greater than 2 mm; spear guiding ring usually double (Fig. 7 L): *Laimydorus*
(very close or identical to *Mesodorylaimus*)

22(12) Cephalic setae present (Fig. 7 M); cuticle with longitudinal ridges and very coarse annulation (Fig. 7 M): *Atylenchus*
Cephalic setae absent; cuticular ridges lacking; cuticle annulation not coarse 23

23(22) Extra, loose outer cuticle layer; spear long, approximately same length as esophagus (Fig. 8 A): *Hemicycliophora*
No extra loose cuticle layer; spear fairly short and not nearly as long as esophagus 24

24(23) Tail filiform (Fig. 8 B); esophagus not overlapping intestine, and median bulb of esophagus small and ovate (Fig. 8 C): *Tylenchus*
Tail elongate conical but not filiform (Fig. 3 B; 8 F); esophagus overlapping intestine, and median bulb of esophagus prominent and spherical (Fig. 2 A, B) 25

25(24) Spear slender with or without modified basal knobs (Fig. 8 H); single ovary anterior to posterior vulva (Fig. 8 I); bursa lacking (Fig. 8 J): *Aphelenchoides*
Spear stout with large basal knobs (Fig. 8 D); two ovaries, one anterior and one posterior to median vulva (Fig. 8 G); bursa present (Fig. 8 E): *Hirschmanniella*

26(11) Spinneret present (Fig. 3 D, E, F) 27
Spinneret lacking or obscure 44

27(26) Esophagus with basal bulb (Fig. 2 E, F, G) 28
Esophagus cylindrical with no basal bulb (Fig. 2 J, L) 37

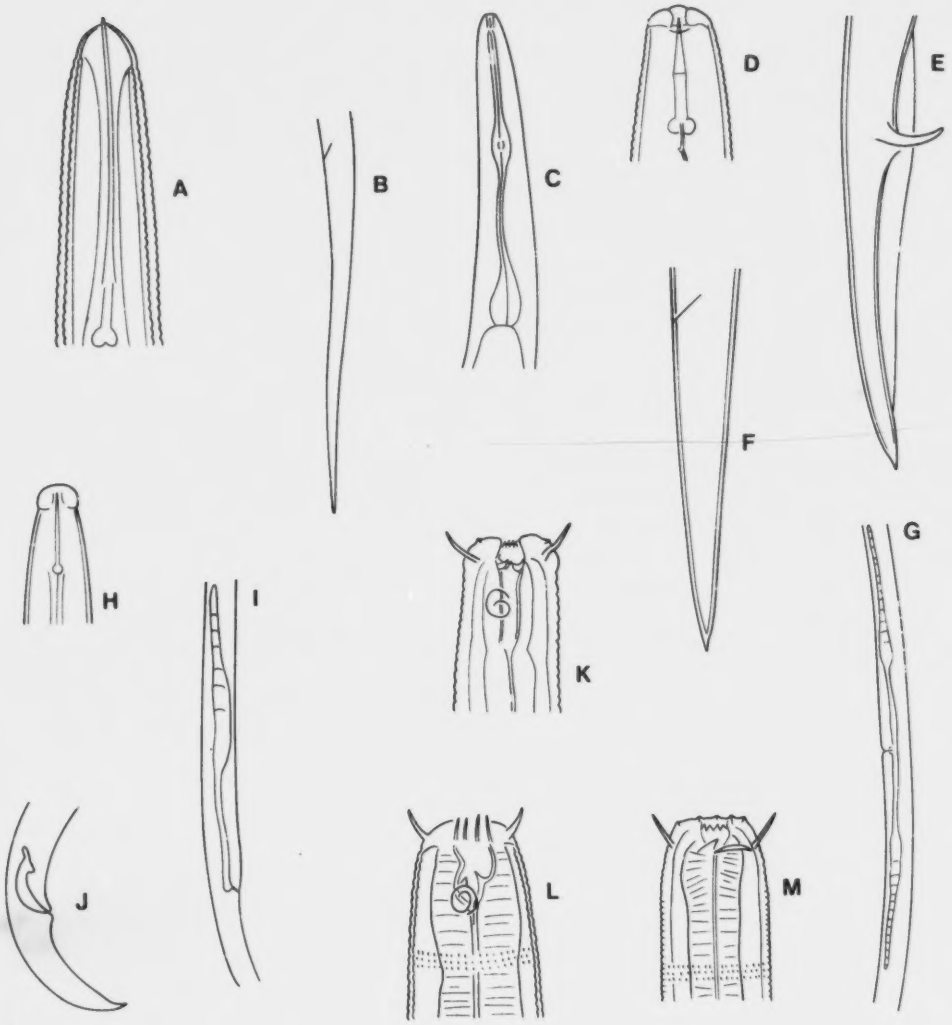

Fig. 8 - A, head *Hemicycliophora vidua* (X 500); B, female tail *Tylenchus exiguus* (X 500); C, anterior part *Tylenchus* sp. (X 500); D, head *Hirschmanniella* sp. (X 1000); E, male tail *Hirschmanniella* sp. (X 500); F, female tail *Hirschmanniella* sp. (X 500); G, female gonads *Hirschmanniella* sp. (X 100); H, head *Aphelenchoides sacchari* (X 1000); I, female gonad *Aphelenchoides* sp. (X 250); J, male tail *Aphelenchoides sacchari* (X 500); K, head *Ethmolaimus* sp. (X 1000); L, head *Achromadora* sp. (X 1000); M, head *Chromadorita leuckarti* (after Micoletzky, 1925).

28(27) Amphid multispiral (Fig. 1 A), slit-like (Fig. 1 E) or obscure 29
Amphid large, unispiral, circular or open circle (Fig. 1 B, C, D) 34

29(28) Amphid spiral 30
Amphid slit-like or obscure 31

30(29) Dorsal tooth and opposing subventral teeth large; tubular section of stoma extending posterior to teeth (Fig. 8 K): *Ethmolaimus*
Dorsal tooth large, and opposing subventral teeth small or obscure; stoma ending just posterior to teeth; tubular section posterior to teeth lacking (Fig. 8 L): *Achromadora*

31(29) Stoma with large sharp dorsal tooth and obscure subventral teeth (Fig. 8 M); cuticle marked with punctations: *Chromadorita*
Stoma with 2 or 3 small teeth or no teeth; cuticle lacking punctations 32

32(31) Cephalic setae present; cuticle distinctly annulated (Fig. 9 A): *Anaplectus*
Cephalic setae absent; cuticle weakly annulated or not annulated 33

33(32) Inconspicuous peg-like spinneret (Fig. 9 D); stoma funnel-shaped (Fig. 9 B); basal bulb of esophagus lacking valves (Fig. 9 C): *Monochromadora*
Prominent long conical spinneret (Fig. 9 I); stoma tubular (Fig. 9 G); basal bulb of esophagus with valves (Fig. 9 H): *Rhabdolaimus*

34(28) Basal bulb of esophagus divided transversely in two places (Fig. 9 E); anterior end of of body set off by shallow construction at level of base of stoma (Fig. 9 F): *Prodesmodora*
Basal bulb of esophagus not divided transversely; anterior end of body not set off, or set off at base of lips 35

35(34) Cuticle not annulated (Fig. 10 A); single ovary anterior to posterior vulva (Fig. 10 B): *Monhystrella*
Cuticle annulated; two ovaries, one anterior and one posterior to median vulva (Fig. 10 E) 36

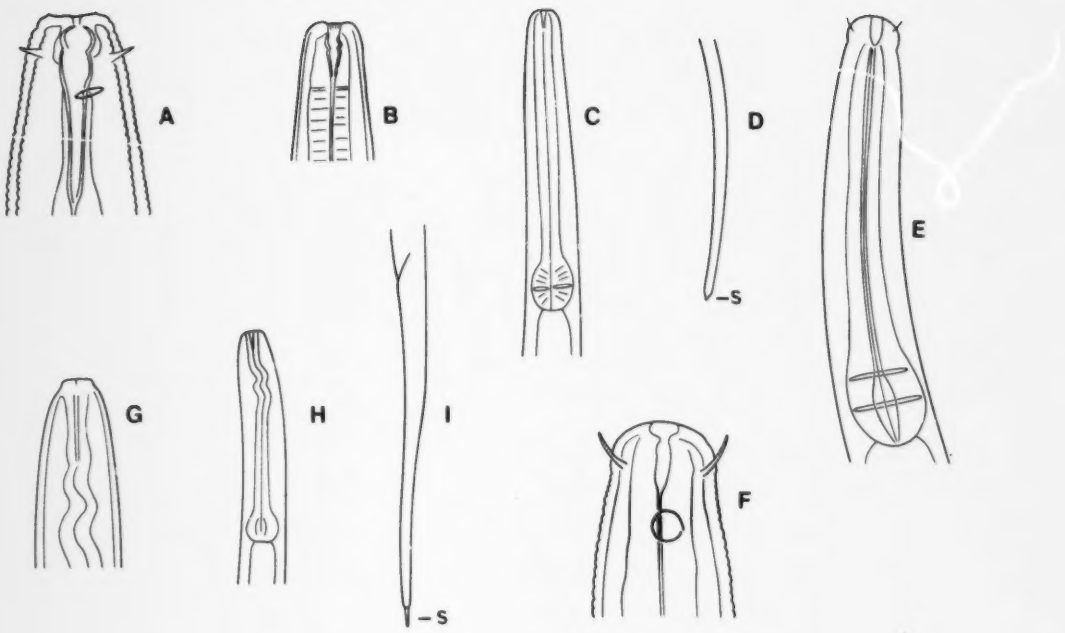

Fig. 9 - A, head *Anaplectus similis* (X 1000); B, head *Monochromadora* sp. (X 1000); C, anterior part *Monochromadora* sp. (X 500); D, tail terminus *Monochromadora* sp. (X 500); E, anterior part *Prodesmodora* sp. (X 500); F, head *Prodesmodora* sp. (X 1000); G, head *Rhabdolaimus* sp. (X 1000); H, anterior part *Rhabdolaimus* sp. (X 500); I, female tail *Rhabdolaimus* sp. (X 500) *s*=spinneret.

36(35) Cephalic setae absent; stoma cylindrical (Fig. 10 C); tail moderately clavate (Fig. 10 D): *Leptolaimus*
Cephalic setae present; walls of stoma converging slightly posteriorly (Fig. 10 J); tail not clavate or slightly clavate (Fig. 10 H, I): *Plectus*

37(27) Spinneret located ventrally on tail (Fig. 5 C); stoma with large subventral tooth (Fig. 5 B): *Mononchulus*
Spinneret on tail terminus; no large tooth or large tooth dorsal in stoma 38

38(37) Anterior end of body fairly narrow and rounded (Fig. 10 G; 11 A) 39
Anterior end of body broad and square (Fig. 11 D, G) 41

39(38) Stoma distinct, elongate, and cylindrical; weak cuticle annulations (Fig. 10 G); single ovary (Fig. 10 F): *Cylindrolaimus*

Stoma a short broad cylinder or obscure; fairly strong cuticle annulation; two ovaries 40

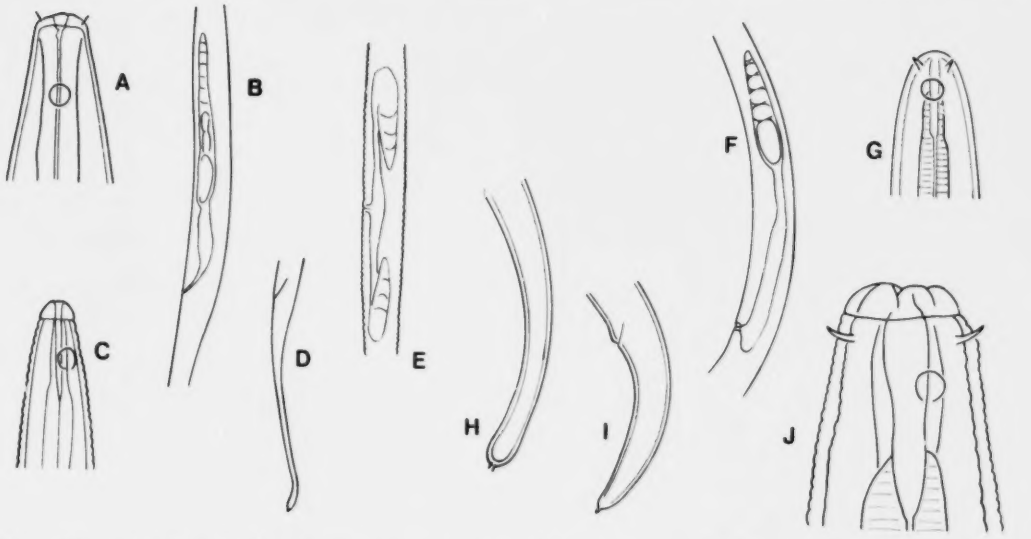

Fig. 10 - A, head *Monhystrella* sp. (X 1000); B, female gonad *Monhystrella* sp. (X 250); C, head *Leptolaimus* sp. (X 1000); D, female tail *Leptolaimus* sp. (X 250); E, female gonads *Leptolaimus* sp. (X 500); F, female gonad *Cylindrolaimus* sp. (X 250); G, head *Cylindrolaimus* sp. (X 1000); H, tail terminus *Plectus* sp. (X 600); I, female tail *Plectus* sp. (X 600); J, head *Plectus* sp. (X 1000).

40(39) Stoma obscure; amphid circular and prominent (Fig. 11 A): *Aphanolaimus*

Stoma a short broad cylinder; amphid unispiral and prominent (Fig. 11 B): *Paraphanolaimus*

41(38) Amphid multispiral; stoma with large dorsal tooth (Fig. 11 C): *Paracyatholaimus*

Amphid stirrup-shaped (Fig. 1 F), circular (Fig. 1 C) or obscure; dorsal tooth lacking or inconspicuous 42

42(41) Amphid circular (Fig. 11 D); single outstretched ovary anterior to posterior vulva (Fig. 11 E): *Monhystera*

Amphid stirrup-shaped or obscure; two ovaries, one anterior and one posterior to median vulva (Fig. 11 H) 43

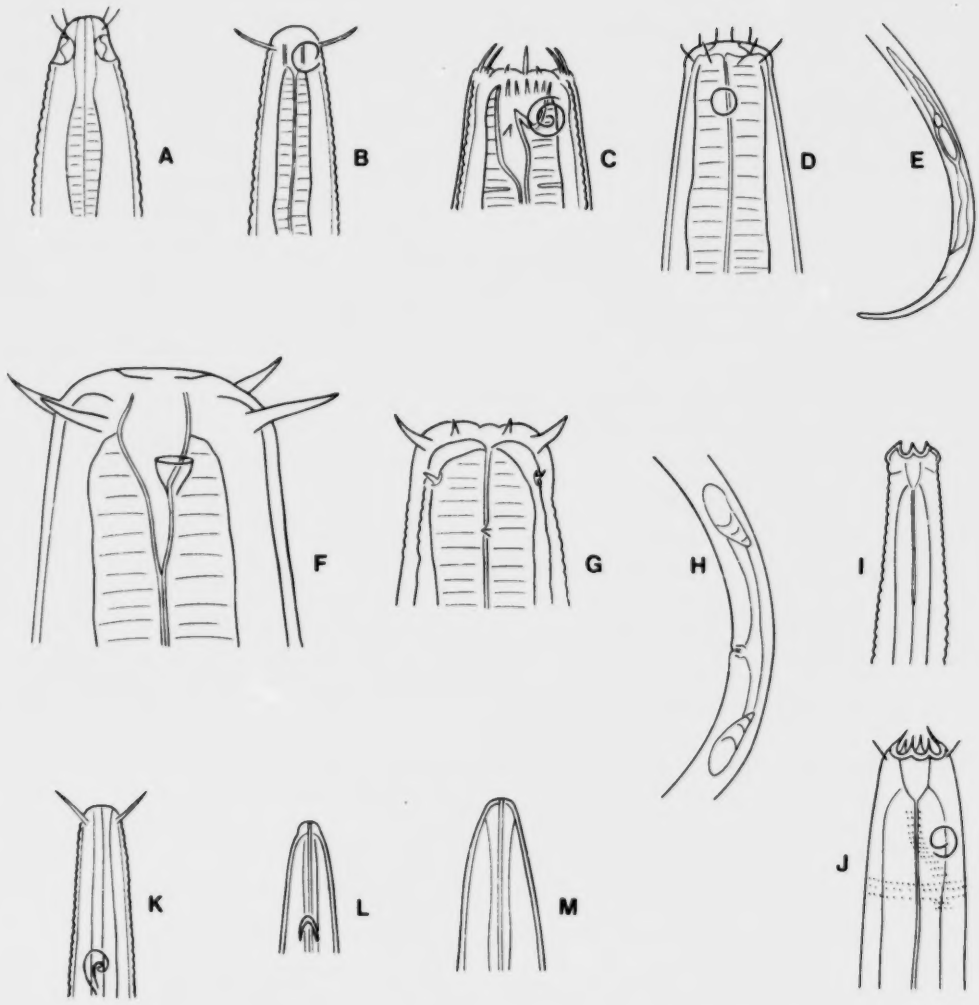

Fig. 11 - A, head *Aphanolaimus* sp. (X 1000); B, head *Paraphanolaimus* sp. (X 1000); C, head *Paracyatholaimus truncatus* (after Cobb, 1914); D, head *Monhystera* sp. (X 1000); E, posterior part *Monhystera* sp. (X 100); F, head *Tobrilus* sp. (X 1000); G, head *Tripyla* sp. (X 1000); H, female gonads *Tripyla* sp. (X 100); I, head *Teratocephalus* sp. (X 1000); J, head *Euteratocephalus* sp. (X 1000); K, head *Bastiania exilis* (X 1000); L, head *Amphidelus hortensis* (X 1000); M, head *Alaimus primitivus* (X 1000).

43(42) Stoma funnel-shaped; amphid stirrup-shaped (Fig. 11 F): *Tobrilus*
Stoma obscure, narrow; amphid obscure (Fig. 11 G): *Tripyla*

44(26) Six sharply pointed, strongly cuticularized, incurved lips (Fig. 11 I, J) 45
Lips not pointed, strongly cuticularized, nor incurved 46

45(44) Cuticle strongly annulated; amphid obscure; single ovary (Fig. 11 I): *Teratocephalus*
Cuticle marked only by punctations; amphid unispiral (Fig. 11 J); two ovaries: *Euteratocephalus*

46(44) Stoma obscure, slender, weakly cuticularized 47
Stoma not slender or if slender then distinctly cuticularized 51

47(46) Anterior end of body square; small tooth located dorsally in stoma (Fig. 11 G): *Tripyla*
Anterior end of body rounded; no tooth in stoma 48

48(47) Long cephalic setae; distinct cuticular annulation (Fig. 11 K): *Bastiania*
No cephalic setae; no cuticular annulation or very faint annulation 49

49(48) Enlarged basal portion of esophagus sharply set off (Fig. 7 H); faint cuticularized ribs surrounding stoma (Fig. 7 I): *Aulolaimoides*
Basal portion of esophagus enlarges gradually (Fig. 2 K); no faint ribs around stoma 50

50(49) Amphid large and slit-like or crescent-shaped (Fig. 11 L): *Amphidelus*
Amphid obscure (Fig. 11 M): *Alaimus*

51(46) Esophagus cylindrical, lacking basal bulb (Fig. 2 H, I, J, L) 52
Esophagus with basal bulb (Fig. 2 D; 13 C; 14 A) 54

52(51) Three large, hook-like teeth at anterior end of stoma (Fig. 12 A); two ovaries, one anterior and one posterior to median vulva (Fig. 12 B): *Ironus*
No teeth at anterior end of stoma; single ovary 53

53(52) Terminus of tail bluntly rounded (Fig. 12 C); circular amphid (Fig. 10 G): *Cylindrolaimus*
Terminus of tail pointed (Fig. 12 E); amphid stirrup-shaped (Fig. 12 D): *Cryptonchus*

54(51) Basal bulb of esophagus non-muscular and lacking valves; median bulb present (Fig. 2 D).Diplogasteridae . 55
[Several genera in this family, in addition to those included in this key, may occasionally be found in aquatic habitats. See Goodey (1963) for illustrations.]
Basal bulb of esophagus muscular and valvate; median bulb present or absent 56

55(54) Stoma moderately broad and deep; anterior edge of stoma bearing rib-like structures; slender tubular section of stoma extending posterior to large tooth in stoma (Fig. 4 D); amphipds obscure: *Mononchoides*
Stoma slender, barrel-shaped or tubular; anterior edge of stoma without rib-like structures; without tooth in stoma; large oval amphipds (Fig. 12 F): *Goffartia*

56(54) Amphid large and circular 57
Amphid small and slit-like or pore-like 58

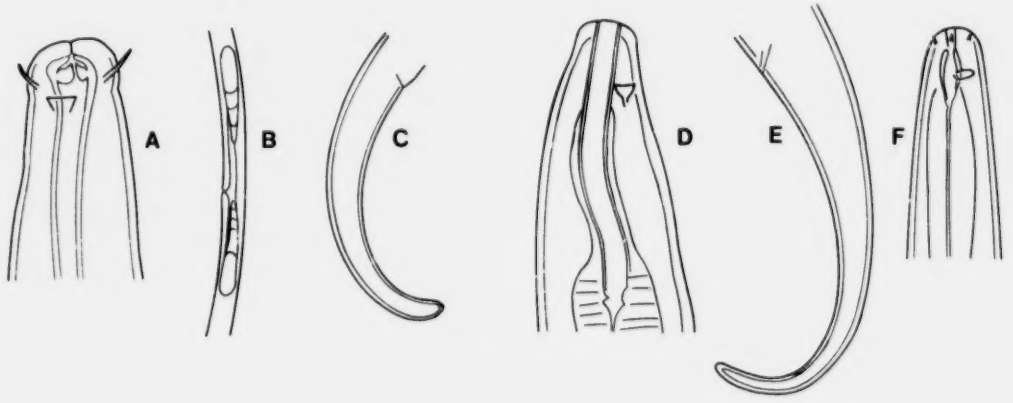

Fig. 12 - A, head *Ironus* sp. (X 1000); B, female gonads *Ironus* sp. (X 100); C, female tail *Cylindrolaimus* sp. (X 600); D, head *Cryptonchus* sp. (X 1000); E, female tail *Cryptonchus* sp. (X 500); F, head *Goffartia* sp. (X 1000).

57(56) Stoma elongate, nearly cylindrical, and strongly cuticularized (Fig. 13 A); two ovaries, one anterior and one posterior to median vulva: *Odontolaimus*
Stoma fairly short, funnel-shaped, and moderately cuticularized (Fig. 10 A); single ovary anterior to posterior vulva (Fig. 10 B): *Monhystrella*

58(56) Cephalic setae present (Fig. 13 B); esophagus uniform in thickness anterior to basal bulb (Fig. 13 C); body slender and tapering very gradually anteriorly and posteriorly: *Chronogaster*

Cephalic setae absent; esophagus narrows between anterior portion and basal bulb (Fig. 2 C; 14 A); body spindle-shaped, considerably thicker in middle than at anterior and posterior ends 59

59(58) Stoma elongate, open, cylindrical (Fig. 13 D); two ovaries, one anterior and one posterior to median vulva; or single ovary anterior to posterior vulva; cuticle weakly annulated: Rhabditidae
[This family is mainly terrestrial, but includes several genera which may occasionally be found in aquatic habitats. See Fig. 13 D for *Rhabditis* sp. and Goodey (1963) and Thorne (1961) for illustrations of additional genera.]

Stoma with small widely separated plates at anterior end followed by a slender moderately cuticularized tube (Fig. 13 E; 14 E); median vulva; single ovary directed first anterior to vulva then reflexed and extending posterior to vulva; cuticle distinctly annulated 60

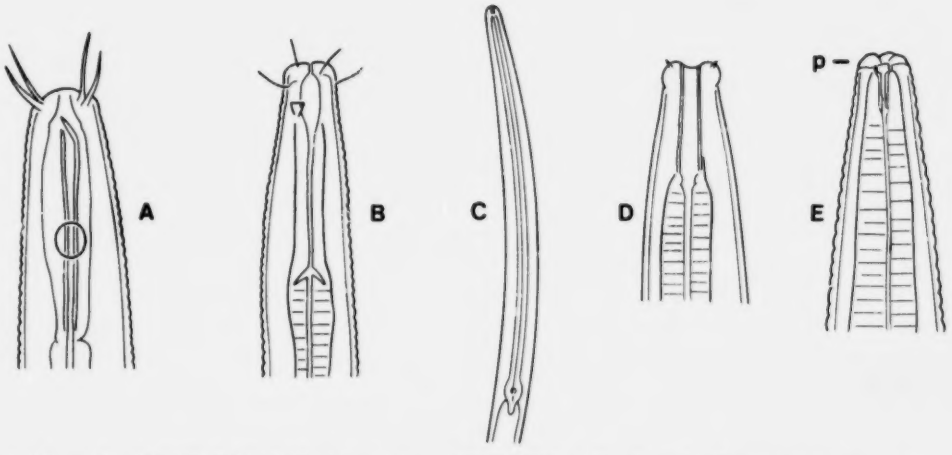

Fig. 13 - A, head *Odontolaimus* sp. (X 1000); B, head *Chronogaster* sp. (X 1000); C, anterior part *Chronogaster* sp. (X 250); D, head *Rhabditis* sp. (X 1000); E, head *Acrobeloides nannus* (X 1000) *p*=probolae.

60(59) Three cuticularized plates or probolae extending beyond lip region (Fig. 13 E); esophagus broadly expanded in middle (Fig. 14 A); tail blunt (Fig. 14 B): *Acrobeloides*

No cuticularized plates or probolae extending beyond lip region; esophagus not expanded in middle; tail blunt or pointed 61

61(60) Tail pointed (Fig. 14 C, D): *Eucephalobus*

Tail blunt (Fig. 14 E, F): *Cephalobus*

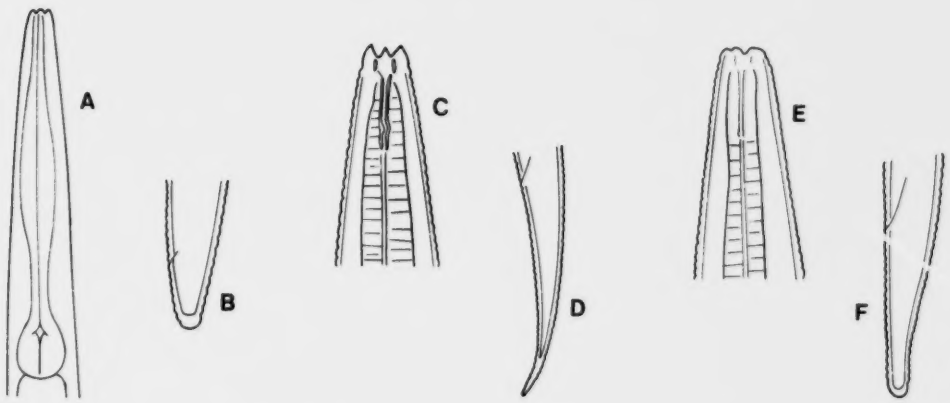

Fig. 14 - A, anterior part *Acrobeloides* sp. (X 500); B, female tail *Acrobeloides nannus* (X 600); C, head *Eucephalobus oxyuroides* (X 1000); D, female tail *Eucephalobus oxyuroides* (X 600); E, head *Cephalobus* sp. (X 1000); F, female tail *Cephalobus* sp. (X 500).

SECTION III

CLASSIFICATION OF GENERA INCLUDED IN KEY

Phylum NEMATODA

Class ADENOPHOREA

Chromadorida	Chromadorina	*Chromadorita* *Ethmolaimus*
	Cyatholaimina	*Achromadora* *Monochromadora* *Paracyatholaimus*
Araeolaimida	Araeolaimina	*Anaplectus* *Anonchus* *Aphanolaimus* *Bastiania* *Chronogaster* *Cylindrolaimus* *Euteratocephalus* *Leptolaimus* *Paraphanolaimus* *Plectus* *Teratocephalus*
Monhysterida	Monhysterina	*Monhystera* *Monhystrella* *Odontolaimus*
Desmodorida	Desmodorina	*Prodesmodora*
Enoplida	Enoplina	*Cryptonchus* *Ironus* *Prismatolaimus* *Rhabdolaimus* *Tobrilus* *Tripyla*
Dorylaimida	Dorylaimina	*Aulolaimoides* *Dorylaimus* *Eudorylaimus* *Labronema* *Laimydorus* *Mesodorylaimus* *Oxydirus* *Nygolaimus* *Paractinolaimus* *Thornia*

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	Alaimina	*Alaimus* *Amphidelus*
Mononchida	Mononchina	*Anatonchus* *Miconchus* *Mononchus* *Mylonchulus* *Prionchulus*
	Bathyodontina	*Mononchulus*
Class SECERNENTEA		
Tylenchida	Tylenchina	*Atylenchus* *Hemicycliophora* *Hirschmanniella* *Tylenchus*
	Aphlenchina	*Aphelenchoides*
Rhabditida	Rhabditina	*Acrobeloides* *Butlerius* *Cephalobus* *Eucephalobus* *Goffartia* *Mononchoides* *Rhabditis*

SECTION IV

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SECTION VI

GLOSSARY

amphid -- one of a pair of organs that open laterally on either side of the anterior end of the body (Fig. 1).

annulations -- transverse grooves circling the body externally in the cuticle at regular intervals (Fig. 14 B-F).

anterior -- toward the front of the body.

basal bulb -- enlargement of the esophagus at the posterior end of the esophagus (Fig. 2 E).

cephalic setae -- bristle-like, elongate cuticular structures at the anterior end of the body (Fig. 4 A, B).

clavate -- club-shaped.

cuticle -- non-cellular external covering of the body; also lining certain structures such as the stoma.

denticles -- small teeth located in stoma (Fig. 6 I).

dorsal -- top side of the nematode body; side of body opposite the side bearing the anus and vulva.

esophagus -- muscular tube leading from the stoma to the intestine (Fig. 2).

filiform -- very slender and thread-like (Fig 3 A shows a filiform tail).

guiding ring -- cuticularized ring surrounding the spear (Fig. 7 A, J, L).

lips -- six (or three) lobes arranged radially around the anterior stomal opening (Fig. 14 C).

median bulb -- enlargement of esophagus approximately midway between the anterior and posterior ends of the esophagus (Fig. 2 A, B).

ovary -- the reproductive gland of the female, often paired, which produces the ova (Fig. 10 B, E, F).

papillae -- minute nipple-like projections of cuticle on surface of body (Fig. 5 A for papillae on lips).

posterior -- toward the back of the body or the tail.

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punctations -- small pits or depressions in the cuticle, usually round (Fig. 8 L, M).

spear -- a hollow, elongate structure in the stoma used to puncture and feed on various food sources (Fig. 2 A; 7 A).

spicules -- male intromittent organs, usually paired, and extrusible through the cloacal opening (Fig. 7 K).

spinneret -- a single duct opening externally on the tail; usually well cuticularized (Fig. 3 D - F; 5 C).

stoma -- the mouth cavity anterior to the esophagus.

subventral -- on either side of the ventral portion of the body.

tail -- portion of body posterior to the anus (Fig. 3).

tooth -- pointed cuticular projection of stoma wall (Fig. 4 C, D; 5 A, B, D).

ventral -- bottom side of nematode body on which the vulva and anus are located.

vulva -- female genital opening (Fig. 10 B, E, F).

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